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SHORT COMMUNICATION

Induced ovule-to-flower switch by interfering with SlIMA activity in tomato

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ABSTRACT

The INHIBITOR OF MERISTEM ACTIVITY in tomato (SlIMA) and MINI ZINC FINGER 2 in Arabidopsis (AtMIF2), two members of the MINI ZINC FINGER family (MIF), are involved in the regulation of flower and ovule development. MIF proteins possess a unique non-canonical zinc-finger domain that confers the capacity to interact with other protein partners. The characterization of SlIMA and AtMIF2 gain- and loss-of-function transgenic lines in *Solanum lycopersicum* and *Arabidopsis thaliana* respectively, allowed the demonstration of their conserved functional role in the termination of floral stem cell maintenance. During early floral development, the expression of Sl*IMA* and At*MIF2* is induced by the MADS-Box transcription factor AGAMOUS (AG). Then, SlIMA or AtMIF2 protein recruits the C_2H_2 zinc finger KNUCKLES (KNU), in a transcriptional repressor complex together with TOPLESS (TPL) and HISTONE DEACETYLASE19 (HDA19). This complex binds to the *WUSCHEL* (*WUS*) locus leading to its repression. To further characterize the role of these interactions in flower development, we have investigated the effects of a dominant negative form of SlIMA, SlIMA^{ch} that leads to spectacular phenotypes, including ovule conversion into a floral meristem.

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Text

The continuous growth and development of plants originate from the activity of the shoot apical meristem (SAM). SAM activity is insured by the maintenance of stem cell activity providing to this structure its indeterminate status. On the opposite, the floral meristem (FM) is a determinate structure producing a limited number of floral organs due to the arrest in stem cell proliferation during the process called FM termination.

As demonstrated in *Arabidopsis thaliana*, the disruption of stem cell maintenance is mainly due to the repression of *WUSCHEL* (*WUS*) which specifies the stem cell identity.[1] The MADS-box transcription factor AGAMOUS (AG) plays a central role in this repression. First, AG directly represses *WUS* during the early stages of FM termination,[2] and next AG acts indirectly through the parallel activation of the expression of two genes, encoding a transcription factor belonging to the C_2H_2 zinc-finger protein family named KNUCKLES (KNU),[3,4] and a MINI ZINC FINGER protein named MIF2.[5] To ensure the complete repression of *WUS*, MIF2 recruits KNU to form a transcriptional repressor complex, together with TOPLESS (TPL) and HISTONE DEACETYLASE19 (HDA19), which binds to the *WUS* locus leading to its complete repression. We recently demonstrated the conservation of this AG-KNU-MIF2-WUS pathway between Arabidopsis and tomato.[2]

In addition to this function in FM termination, the tomato MINI ZINC FINGER protein SlIMA (for *Solanum lycopersicum* INHIBITOR OF MERISTEM ACTIVITY) is also involved in ovule development.[6] Indeed, the up-regulation of SlIMA results in an activation of the expression of the D-function gene *TAgl11*, leading to an increased number of ovules. Interestingly the loss-of function of SlIMA produced undifferentiated structures giving rise to finger-shaped ovules, as the result of a maintained cell proliferation which is defined molecularly by the maintenance of *WUS* expression.

A chimeric SlIMA protein acts as a dominant negative form of SlIMA and induces a conversion of the ovules into floral buds

In this report we aimed at investigating further the effect of the loss-of-function of SlIMA on flower and fruit development in tomato. We generated transgenic tomato plants in which a chimeric version of SlIMA (SlIMA^{ch}) is expressed ectopically. These plants referred to as *35S::SlIMA^{ch}* lines harbor a transgene where the encoded distal half part of the protein was replaced by a mock peptide sequence. As a result the chimeric sequence only harbored the first sub-domain of the C_5H_3 zinc finger domain (Figure 1A). While SlIMA^{ch} conserved its ability to interact with the full length SlIMA protein (Figure 1B), we could not observe any interaction between SlIMA^{ch} and SlKNU, suggesting that the necessary interaction between SlIMA and SlKNU to recruit chromatin repressor complexes as to control the *WUSCHEL* expression[2] may be impaired in the *35S::SlIMA^{ch}* lines.

The expression of the chimeric *SlIMA^{ch}* transgene induced the spectacular conversion of the ovules into floral buds (Figure 1C-D-E) with developing sepals, petals, stamens and carpels (Figure 1D-E). Ectopic flower buds were able to

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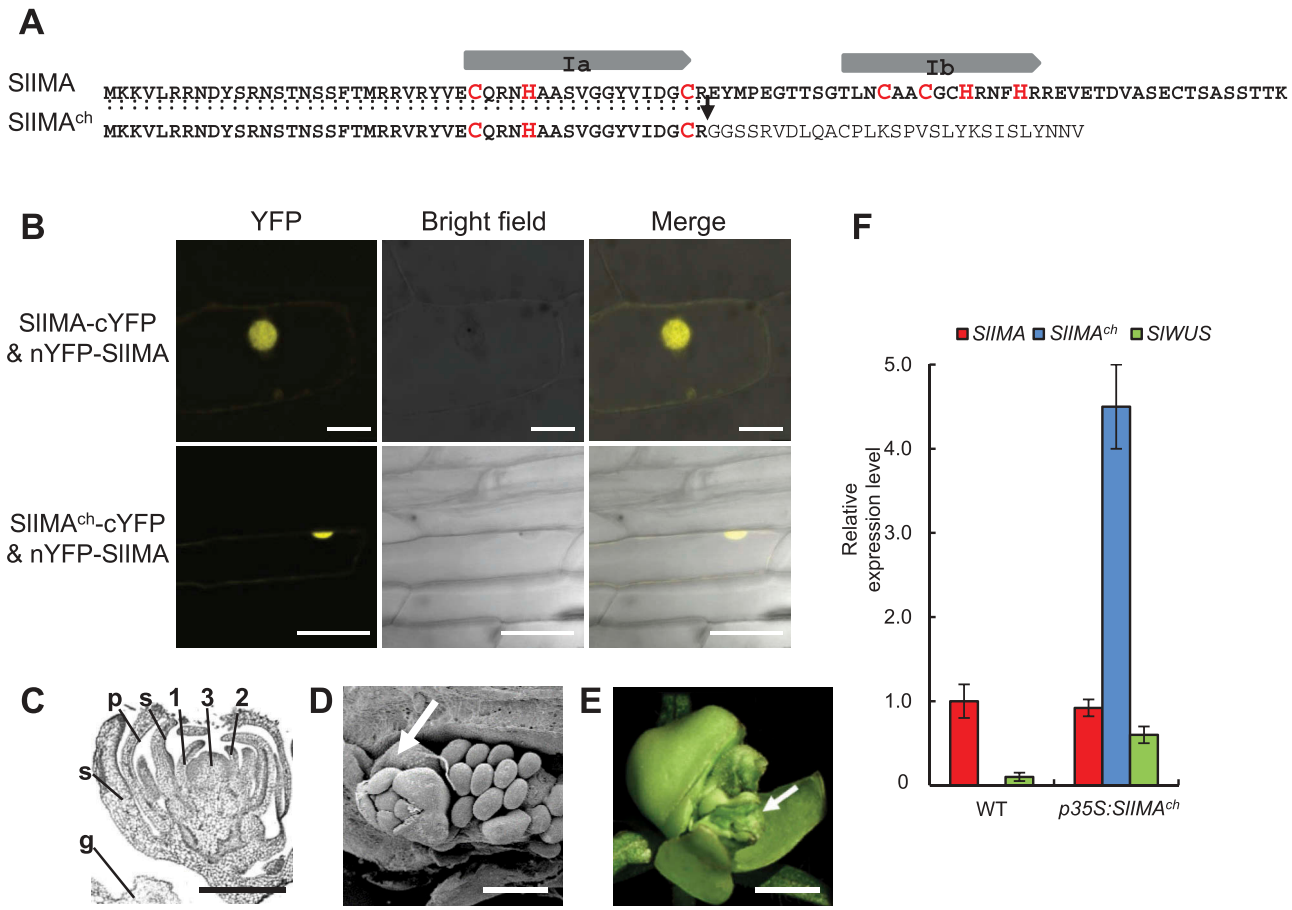

Figure 1. Characterization of SlIMA^{ch} overexpressing plants.

(A) Primary sequence of the chimeric SlIMA protein (SlIMA^{ch}) as compared to the WT SlIMA protein.The position of the mock peptide sequence insertion in SlIMA^{ch} is indicated by an arrowhead. Cysteine and Histidine residues of the two parts of the C_5H_3 zinc-finger domain (Ia and Ib) are highlighted. Note that the second subdomain has been substituted in the SlIMA^{ch} protein.
(B) Interaction analyses by BiFC in onion epidermal cells. Upper panels, SlIMA self-interaction and lower panels, interaction between SlIMA^{ch} and SlIMA. Bars, 25µm.
(C) Cross-section of a floral bud developing inside a *35S::SlIMA^{ch}* fruit (s: sepal; p: petal; e: stamen; g: gel; 1,2,3: carpel primordia). Bars, 100µm.
(D) Scanning electron micrograph of a dissected ovary from *35S::SlIMA^{ch}* transgenic plant. Note the floral meristem (white arrow) deriving from the ovules. Bars, 500µm.
(E) Dissected *35S::SlIMA^{ch}* transgenic fruit showing the matriochka phenotype, i.e. development of an ectopic stage-14 floral bud (white arrow) inside the fruit. Bars, 5mm.
(F) Relative abundance of *SlIMA*, chimeric *SlIMA^{ch}* and *SlWUS* transcripts in *35S:SlIMA^{ch}* transgenic plants compared to wild-type (WT) plants, as measured by RT-qPCR.

develop almost normally inside a fruit, and could even display an iterative "Matriochka-Russian-doll" phenotype: namely ectopic flower buds inside their own carpels, and so on. Interestingly, this "flower-in-fruit" phenotype observed in SlIMA^{ch}–overexpressing tomato is very similar to the phenotype observed in the *knu* mutant in Arabidopsis.[5]

In the *35S::SlIMA^{ch}* lines, the expression of the endogenous Sl*IMA* gene was normal (Figure 1F). Therefore the chimeric version of SlIMA may act as a dominant-negative mutant protein, because its high expression induces much more severe modifications in the ovule development than the sole decrease of Sl*IMA* expression as observed in transgenic tomato lines harboring an antisense RNA or a RNA interference (RNAi) construct.[1] Consistent with this strong effect of a dominant negative protein, the abundance of *SlWUS* mRNA was higher in flower buds of *35S::SlIMA^{ch}* plants compared to that in WT plants (Figure 1F), suggesting that the endogenous SlIMA in the presence of SlIMA^{ch} is not able to participate in the repression of Sl*WUS*.

Altogether these data indicate that the high level of expression of *SlIMA^{ch}* in the *35S::SlIMA^{ch}* lines is likely to sequester the endogenous SlIMA protein (since they are able to interact as shown in Figure 1B), thus preventing its adaptor function required by SlKNU for recruiting the chromatin remodeling proteins to repress *SlWUS*.

Conclusion and perspectives

MINI ZINC FINGER proteins are key components of hormonal signaling and developmental pathways as shown from our work and others.[5–7] They are involved in the FM termination pathway, which ultimately influences the determination of carpel number, but also in ovule development. Yet the precise molecular mechanisms by which MIF proteins can act as adaptor proteins remain to be elucidated.

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